FURTHER STUDIES ON BACTERIUM ABORTUS AND RELATED BACTERIA

III. BACTERIUM ABORTUS AND RELATED BACTERIA IN COW'S MILK

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INTRODUCTION

In any consideration of the infectiousness of a food substance, the number of virulent contaminating organisms is an important factor. In the study reported in this paper, the methods were designed to give results as to the numbers as well as kinds of bacteria of the type of Bacterium abortus and related strains found in freshly drawn milk. The methods were practically the same as those employed in an earlier work of which this is a continuation. The data presented in this paper are therefore supplemental to the earlier report.[1]

It was reported in the earlier paper that besides the typical virulent strains of Bact. abortus, other bacteria of similar morphology were eliminated from the udders of many cows whose milk was examined. The history of those cows was not known. Many other samples of milk have been examined from cows whose history is known in order to determine whether there might be any correlation between the presence of organisms similar to Bact. abortus in the milk and the occurrence of abortion. The fact that the serum of a certain small percentage of aborting cows does not react on the typical strains of Bact. abortus suggested the possibility that some of these bacteria may sometimes be responsible for abortion.

The method of obtaining cultures was as follows: The aseptically drawn milk was plated on plain infusion agar and on infusion agar to which 10% of sterile raw cow's blood serum had been added. After 4 or 5 days' incubation at 37 C. a sufficient number of colonies to represent every kind of colony growing on both sets of plates were fished off and inoculated into litmus whole milk. Usually 10-20 colonies were transferred from each set of plates. A thorough study was made of every strain thus obtained which was morphologically similar to Bact. abortus.

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[1] Jour. Infect. Dis., 1916, 18, p. 437.

The results of the milk examinations are presented in the tables in which 3 types of organisms are considered. Under the heading "Bact. abortus" in the tables there are included only those strains which were agglutinated in high dilutions of Bact. abortus antiserum. Under the heading "Abortus-like bacteria" are included strains which resembled the typical strains in cultural characteristics as well as in morphology, but which were agglutinated only in low dilutions of Bact. abortus antiserum. Under the heading "Bact. lipolyticus" are included the fat decomposing strains described in the earlier publication, and under this heading there are also included strains which resemble Bact. lipolyticus culturally, but which vary slightly in their biochemical reactions.

BACT. ABORTUS AND RELATED BACTERIA IN THE MILK OF COWS WHICH HAVE NOT ABORTED

The numbers of Bact. abortus and related bacteria which were found in the milk of 24 cows which had not aborted is given in Table 1. The cows belonged to a herd in which there had been occasional cases

TABLE 1

BACT. ABORTUS AND RELATED BACTERIA IN THE MILK OF COWS OF HERD A THAT HAVE NOT ABORTED

Cow	Number of Bact. Abortus per C c	Abortus-like Bacteria			Bact. Lipolyticus		
		Number per C c	No. of Strains	Names of Strains	Number per C c	No. of Strains	Names of Strains
1	0	0			0		
4	0	100	1	zj	0		
12	0	0			1,600	2	ze, yu
17	0	0			0		
23	0	0			3,800	1	yb
24	0	80	1	xv	140	1	xy
26	0	0			0		
27	0	0			0		
28	0	0			0		
34	0	0			0		
39	0	0			0		
41	0	0			0		
43	0	0			0		
44	0	30	1	yo	0		
48	0	0			0		
51	0	10	1	yl	0		
52	0	0			0		
53	0	0			0		
100	0	0			960	2	zt, zv
106	0	0			0		
107	0	600	2	zp, zq	300	1	zn
114	0	0			0		
206	0	0			500	1	zo
211	0	600	1	zl	0		

of abortion during the few years previous to the examination of the milk samples. Typical strains of Bact. abortus were never cultivated from the milk of the cows which had not aborted, but abortus-like strains were cultivated from the milk of 6, or 25% of them. Bact. lipolyticus was also cultivated from 25% of the cows. If the 2 columns are considered together, bacteria related to Bact. abortus were culti-

TABLE 2

BACT. ABORTUS AND RELATED BACTERIA IN THE MILK OF COWS THAT HAVE ABORTED AS A RESULT OF NATURAL INFECTION

Herd	Cow	Date Abortion Occurred	Agglutination Tests*		Date Milk Was Examined	Bact. Abortus per C c	Abortus-like Bacteria			Bact. Lipolyticus		
			Date	Reaction of Blood Serum			No. per C c	No. of Strains	Names of Strains	No. per C c	No. of Strains	Names of Strains
A	18	7/20/17	11/ 4/16	—	4/30/17	0	0			0		
			10/16/17	+	9/22/17	0	0			4,500	2	zy, aaa
	19	5/ 6/15	11/ 4/16	—								
			10/18/17	—	4/30/17	0	0			4,000	1	yq
			1/25/18	+	9/22/17	0	0			2,000	1	aag
	21	8/11/13	11/15/16	—								
			10/18/17	+								
			11/27/17	+	3/ 2/17	0	0			1,600	1	ya
	33	6/19/15	11/ 4/16	—	3/ 2/17	0	70	2	xp, xq	0		
			10/18/17	+	9/22/17	0	0			0		
	40	8/12/17	11/ 4/16	—								
			10/18/17	+	9/22/17	0	250	1	aae	0		
	45	11/ 2/16	11/ 4/16	+	3/ 2/17	0	400	4	xh, xj, xm, xw	0		
			10/18/17	+	9/22/17	0	0			2,000	2	aai, aaj
	46	7/21/17	11/ 4/16	—	4/30/17	0	0			9,600	2	ym, zd
			10/18/17	+	9/22/17	0	0			2,500	1	aak
	50	10/26/16	11/16/16	+								
			10/18/17	+	3/ 2/17	0	60	2	xf, xn	0		
	109	12/ 2/16	11/ 4/16	—								
			10/18/17	—								
			11/27/18	—	3/ 2/17	0	15	1	xs	0		
B	28016	11/ 6/17	4/28/17	—								
			6/ 4/17	—								
			8/29/17	—								
			11/ 6/17	+	11/10/17	0	0			8,000	1	abg
	27723	10/ 6/17	8/21/16	—								
			6/ 4/17	—	10/10/17	0	130	1	aau	800	2	aas, aat
			10/ 6/17	—								
			10/27/17	—	10/27/17	0	1,600	2	abc, abd	900		
C	317	12/18/16	10/ 1/16	+								
			1/20/17	+ †	5/ 3/17	0	0			120	1	zg
			1/18/18	+	10/ 5/17	0	0			1,800	1	aav

* The agglutination tests recorded in this table, and also those recorded in Tables 3 and 5, were made by the investigators of the Pathological Division, who kindly gave any information in their records which could be correlated with this study.

† The serum from a sample of mixed milk gave a negative reaction in February, 1917, and the serums from the milk samples taken separately from the 4 quarters all gave a negative reaction in December, 1917.

vated from the milk of 10, or 41.7% of the 24 cows. The names of the strains of bacteria related to Bact. abortus are given in Table 1. The characteristics of these strains are given in Table 4. Their characteristics and their relationship to Bact. abortus will be discussed later.

BACT. ABORTUS AND RELATED BACTERIA IN THE MILK OF COWS WHICH HAVE ABORTED

The number of Bact. abortus and related bacteria found in the milk of 12 cows which have aborted as a result of natural infection are given in Table 2. Nine of the cows belonged to the same herd (Herd A) as the cows considered in Table 1. A study of this table brings out 2 significant facts: (1) although the serum reactions of these cows indi-

TABLE 3

BACT. ABORTUS AND RELATED BACTERIA IN THE MILK OF COWS THAT HAVE ABORTED AS A RESULT OF INOCULATION

Cow	Date of Inoculation	Date of Abortion	Agglutination Tests			Date Milk Was Examined	No. of Bact. Abortus per C c	No. of Abortus-like Bacteria per C c	No. of Bact. Lipolyticus per C c
			Blood Serum	Milk Serum					
				Date	Reaction				
50	Numerous inoculations		Continuously positive			2/16/17 5/ 3/17* 10/ 5/17*	25,000 35,000 25,000	0 0 0	25,000 15,000 25,000
51	Numerous inoculations		Continuously positive			5/ 3/17* 10/ 5/17*	145,000 120,000	0 0	0 0
313	10/14/16	1/15/17	Continuously positive	12/20/17	slight	2/16/17 5/ 3/17* 10/ 5/17*	450 0 0	0 0 0	0 30 000 0
314	10/14/16	3/19/17	Continuously positive	12/20/17	strong	5/ 3/17 10/ 5/17	0 0	0 0	0 0

* The cow was dry. A serum-like material from the udder was plated.

cated that at least 10 of the 12 were infected with Bact. abortus, typical strains were never cultivated from any of these samples of milk, and (2) related bacteria were cultivated from 100% of the cows. Abortus-like strains were cultivated from 6, or 50%, of the 12 cows. The numbers of Bact. lipolyticus per c c of milk were noticeably larger in the case of those cows which had aborted, as compared with those which had not aborted, as shown in Table 1.

The prevalence of Bact. abortus and related bacteria in the udders of cows which have aborted as a result of inoculation is shown in Table 3. All 4 cows were inoculated by investigators of the Patho-

logical Division, Bureau of Animal Industry, for purposes of another experiment. Cows 50 and 51 were inoculated many times with a mixture of several strains of Bact. abortus, with the result that their blood serum agglutinated the antigen in higher dilutions than the serum of naturally infected cows. The serum from these cows was used for the agglutination tests recorded in Table 4. Cows 313 and 314 were inoculated only once with the same strains of Bact. abortus as were used for the inoculation of Cows 50 and 51. Every examination of the milk (or serum-like material from the udder in case the cow was dry) of Cows 50 and 51 showed very great numbers of typical Bact. abortus per c c. One month after Cow 313 had aborted the plates showed 450 Bact. abortus per c c of milk. Three months later, when the cow was dry, they had disappeared from the udder. No Bact. abortus could be found in the milk of Cow 314, 1½ months and 6½ months after abortion. Bact. lipolyticus was cultivated from the udders of 2, or 50% of the 4 cows. The numbers of Bact. lipolyticus in the milk or other material from the udder of Cows 50 and 313 were very high. No abortus-like strains were cultivated from the udders of any of these inoculated cows.

GENERAL CONCLUSIONS IN REGARD TO THE PREVALENCE OF VIRULENT STRAINS OF BACT. ABORTUS IN COW'S MILK

The data presented in Tables 1, 2, and 3 offer some suggestions as to the actual numbers of virulent strains of Bact. abortus in cow's milk. It was not surprising that none of these organisms could be cultivated from the milk of cows that had not aborted, but in view of the work of other investigators it was to be expected that if the cultural methods were adequate Bact. abortus could be demonstrated to be in the milk of the cows which had aborted as a result of natural infection. Schroeder and Cotton,[2] Fabyan,[3] and Fleischner and Meyer,[4] determined the presence of Bact. abortus in cow's milk by inoculating guinea-pigs with the milk. They never isolated the organism directly from the milk, and therefore obtained no information concerning the actual number of organisms present. Zwick and Krage[5] cultivated Bact. abortus from cow's milk by centrifuging the milk and streaking the sediment on agar slopes. This method also failed to give informa-

[2] Twenty-Eighth Ann. Report Bur. An. Ind., Department of Agriculture, 1911, p. 139.
[3] Jour. Med. Research, 1913, 28, p. 85.
[4] Am. Jour. Dis. Child., 1917, 14, p. 157.
[5] Berlin Tier. Wchnschr., 1913, 29, p. 41.

tion as to the actual number of organisms present. Cooledge[6, 7] has adopted the agglutinative reaction of the milk for Bact. abortus as a test indicating the distribution of that organism. Such a test, however, not only fails to give information concerning the number of organisms present, but also, in all probability, it frequently indicates udder infection after it no longer exists. Cooledge[6] states that he could not always find the organism by animal inoculation when agglutinins were demonstrated to be in the milk.

Should the fact that virulent Bact. abortus has not been cultivated from the milk of the naturally infected cows be explained by assuming that the method failed to demonstrate them although they were present? The first growth on artificial mediums of Bact. abortus from pathologic material is known to be somewhat difficult to obtain. But the infusion agar containing 10% of raw cow's blood serum which was used for plating the milk is a good medium for the growth of this organism. It seems probable that if there were large numbers of Bact. abortus in the milk, colonies would have appeared on some of the plates. However that may be, the plating method employed was demonstrated to be adequate for the cultivation of those strains which were used for inoculation, as shown in Table 3. Fabyan[8] noted that strains of Bact. abortus which have become adapted to growth on artificial mediums may be passed through animals and recovered from the lesions more readily than strains cultivated from pathologic material for the first time. The investigators of the Pathological Division report that they have had the same experience. It may be that the reason that Bact. abortus could be cultivated from the milk of the inoculated cows was because those strains had previously become adapted to growth on artificial mediums. At any rate the essential point is that the strains of Bact. abortus used for inoculation could be cultivated from the milk. The heavy infection of the udders of Cows 50 and 51 cannot be compared with natural conditions, for these cows were heavily inoculated again and again. Since Cows 313 and 314 were inoculated with the same strains as Cows 50 and 51, we can assume that if Bact. abortus were present in considerable numbers in their udders it could have been demonstrated as well as in the case of Cows 50 and 51. It was demonstrated in the milk of Cow 313 one month after abortion occurred, but could not be cultivated the 3d and

[6] Jour. Agri. Research, 1916, 5, p. 871.
[7] Ibid., 1917, 37, p. 207.
[8] Ibid., 1912, 26, p. 441.

8th months later, although the blood serum continued to give a positive reaction. Unfortunately no examinations were made of the milk of Cow 314 until about 1½ months after abortion, when Bact. abortus had apparently disappeared from the milk, although in this case also the blood serum gave a positive agglutination reaction. However, Bact. abortus may have been present in such small numbers that it failed to be isolated. An example of this possibility is the presence of Strain yl in the milk of Cow 51 of Herd A (Table 1) in such small numbers that the result of plating showed only 10 per c c. Since a 1 : 10 dilution was the lowest made, the chances are that an organism present in such small numbers would not grow in the plate culture, whereas the inoculation of milk containing 10 virulent Bact. abortus per c c might give a positive result.

The results obtained by the plating of milk samples indicate that virulent strains of Bact. abortus are not continuously eliminated in considerable numbers in the milk of cows which have been infected, although the blood serum may continue to give a positive agglutination reaction. On the other hand, the persistence of great numbers of Bact. abortus in the udders of Cows 50 and 51 may illustrate the possible numbers that a badly infected cow could excrete in her milk during the height of her infection.

THE PREVALENCE OF ABORTUS-LIKE BACTERIA IN COW'S MILK

Under the heading "Abortus-Like Bacteria" in the tables are included all strains with the morphology and cultural characteristics of Bact. abortus, but which were agglutinated only in low dilutions of Bact. abortus antiserum. From some of the samples of milk, strains were obtained of similar morphology which were not agglutinated in the 1 : 20 dilution — the lowest tested dilution of the serum. Usually such strains were distinctly different from Bact. abortus in their biochemical reactions. The cultures which showed no agglutinative reaction were discarded, and are not considered in this paper, for they were not found in the milk in any considerable numbers. In response to the agglutination test the abortus-like strains varied widely from Strains xs, abd, and xq (Table 4) which were agglutinated in the 1 : 160 dilution, to strain xt, which was agglutinated in the 1 : 20 dilution. The biochemical reactions of the abortus-like strains are given in Table 4, where they may be compared with the reactions of a typical virulent strain (aap). It may be observed that strain xt, which was acted on only in the lowest dilution of Bact. abortus antiserum, and

TABLE 4

CHARACTERISTICS OF BACT. ABORTUS AND RELATED BACTERIA

Type of Organism	Strain	Reaction in Litmus Whole Milk	Fermentation of					Decomposition of		Reaction in		Reduction of Hydrogen-ion Concentration in Broth Cultures Recorded in PH Values*	Agglutination Reactions in Bact. Abortus Antiserum							
			Dextrose	Lactose	Saccharose	Maltose	Mannite	Urea	Asparagin	Nitrate Broth	Gelatin		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560
Bact. abortus	aap	alkaline	—	—	—	—	—	+	+	slight	—	0.7	C	C	C	C	C	C	+	0
Abortus-like strains	abd	no change	—	—	—	—	—	—	+	+	—	1.3		++	++	+	0	0		
	xs	no change	slight	—	—	—	—	—	—	—	—	1.0	C	C	++	+	0	0		
	xq	no change	slight	—	—	—	—	—	—	—	—	0.3		+	+	+	0			
	xh, xn	acid in cream layer, alkaline beneath	slight	—	—	—	—	+	+	slight	—	0.9		++	+	0	0			
	xm	alkaline	slight	—	—	—	—	+	+	—	—	1.0		++	+	0	0			
	xw	no change	slight	—	slight	—	—	+	+	—	—	0.8		+	+	0	0			
	xv, aae	alkaline	—	—	—	—	—	+	+	±	—	1.0		++	0	0	0			
	xj, zj	acid in cream layer, alkaline beneath	—	—	—	—	—	—	—	—	—	0.8		+	0	0	0			
	xf, xp	alkaline	slight	—	—	—	—	—	—	—	—	0.5		+	0	0	0			
	abe	alkaline	slight	—	slight	—	—	—	slight	—	—	0.7		+	0	0	0			
	yl		—	—	—	—	—	—	—	—	—	1.0		+	0	0	0			
	aau	alkaline	—	—	—	—	—	+	+	—	+	2.2		+	0	0	0			
	zl	alkaline	—	—	—	—	—	—	+	—	+	1.0		+	0	0	0			
	abc	alkaline	slight	slight	slight	slight	slight	+	slight	—	+	1.0	+	+	0	0	0			
	xt	alkaline	slight	—	—	—	—	+	+	—	—	1.0	+	0	0	0	0			
Atypical strains of Bact. lipolyticus	zd, ze, zn, zy, ys, aaj, aak	acid in cream layer	—	—	—	—	—	—	—	—	—	0.3		+	0	0	0			
	aat	acid in cream layer	slight	—		slight	—	—	—	—	—	0.3								
	xy, xa, yb, yr, aav	acid in cream layer	slight	slight	—	—	—	±	—	—	—	0.0								
	aag	no change	—	—	—	—	—	+	+	—	—	0.0								
	ym, yu, zv, aai	no change	—	—	—	—	—	—	—	—	—	0.0								
Bact. lipolyticus	zg, zo, zt, aaa, aaq, aas, abg	acid in cream layer	—	—	—	—	—	—	—	—	—	0.0								

* The initial hydrogen-ion concentration of the broth was 6.0 PH.

several of the other strains agree very closely in biochemical reactions with the typical virulent strain of Bact. abortus. Some of the strains vary considerably from the type strain in their biochemical reactions. Some fail to produce alkali in milk; some are able to decompose the fat of the cream layer, in which respect they resemble Bact. lipolyticus. There is a tendency for the abortus-like strains to attack the carbohydrates slightly, with dextrose yielding most readily. Urea and asparagin, are decomposed by about one half of the strains. Three of the strains, aau, zl, and abc, are distinctly atypical in their liquefaction of gelatin. All the abortus-like strains reduced the hydrogen-ion concentration of broth cultures, but there was a considerable variation in the extent of reduction. Strain aau, which gave a reduction of the hydrogen-ion concentration equivalent to $2.2P_H$ is distinctly atypical, for it reacts in this respect with 3 times the vigor of the typical strains. It agrees with Bact. bronchisepticus (Table 1 of the 2d paper of this series)[9] in every respect except that it liquefies gelatin very slightly.

Abortus-like strains were isolated from 6 of 24, or 25%, of the cows which had not aborted (Table 1). They were isolated from 6 of 12, or 50%, of the cows that had aborted as a result of natural infection (Table 2). They were never isolated from the contents of the udders of the inoculated cows.

Judged by the figures resulting from the study of the limited number of samples of milk, abortus-like strains occur in the milk of cows which have aborted with twice the frequency with which they occur in the milk of cows which have not aborted.

The criticism has been suggested that since the serum from only 2 cows was used for the agglutination tests, these cows at some time may have been infected with the abortus-like organisms, and for this reason, and not because the organisms are related to Bact. abortus, positive serologic reactions were obtained. But Cows 50 and 51, which supplied the serum for these tests, were found to be particularly free from infection with abortus-like bacteria (Table 3). Furthermore, it would be unreasonable to assume that any 2 cows would during their lifetime harbor so many varieties of abortus-like bacteria as were shown to be agglutinated by the serum of these 2 cows (Table 4).

In only one case were large numbers of abortus-like bacteria found in the milk. From the milk of Cow 27723, of Herd B (Table 2), 1,600 of these organisms were found per c c. There were 2 strains of

[9] Jour. Infect. Dis., 1918, 22, p. 583.

them, but six sevenths of the number were of Strain abd, which was agglutinated in the 1 : 160 dilution of Bact. abortus antiserum (Table 4, Line 2, under "Abortus-like Bacteria"). The finding of a large number of bacteria of an abortus-like organism in the milk of this cow is of especial interest, for she had aborted 3 weeks before it was isolated from her milk, and on the day of abortion her serum reacted negatively to a suspension of a typical strain of Bact. abortus. It is well in this connection to recall the fact that the agglutinating power of the Bact. abortus antiserum used for the tests recorded in Table 4, was much stronger than that of a naturally infected cow, and, therefore, it might show relationships which would not be made evident by the use of the serum of a naturally infected cow.

COMPARISON OF BACT. ABORTUS AND BACT. LIPOLYTICUS

In the earlier publication[1] a description was given of a fat decomposing bacterium found in cow's milk which agreed with Bang's[10] original description of the organism causing contagious abortion. The first paper of this series[11] shows that Bact. lipolyticus is not pathogenic to guinea-pigs. In morphology it is identical with Bact. abortus, but Bact. lipolyticus is gram-positive, whereas Bact. abortus is gram-negative. In Table 4, the biochemical reactions of typical strains of Bact. lipolyticus can be compared with the reactions of a typical virulent strain of Bact. abortus (aap). The 2 types of organisms are alike in their failure to decompose the carbohydrates and mannite, and in their failure to liquefy gelatin; but they differ markedly in their reaction in several kinds of mediums. In whole milk Bact. lipolyticus produces an acidity by decomposing fat, whereas Bact. abortus produces an alkalinity. Bact. lipolyticus does not decompose urea and asparagin, whereas Bact. abortus does. In broth cultures Bact. lipolyticus grows without changing the hydrogen-ion concentration, whereas Bact. abortus reduces it considerably. In cultural characteristics also the two types of organisms differ distinctly. Bact. lipolyticus does not cloud broth cultures, as does Bact. abortus, but it forms dustlike granules which sink to the bottom of the tube. In agar shake cultures, Bact. lipolyticus grows in a "diaphragm" a few mm. beneath the surface of the agar. Usually scattered colonies extend upward from this diaphragm toward the surface, but there is no visible growth beneath the diaphragm. Years of artificial cultivation do not

[10] Ztschr. Thiermedicin, 1897, 1, p. 241.
[11] Jour. Infect. Dis., 1918, 22, p. 576.

alter this relationship of Bact. lipolyticus to the supply of oxygen. Bact. abortus produces a heavy surface growth, but no colonies appear beneath the surface in agar shake cultures of strains which have adapted themselves to artificial cultivation, although freshly isolated strains require a reduced oxygen tension. On agar slopes Bact. lipolyticus produces an exceedingly faint dry growth in separate colonies, whereas Bact. abortus produces an abundant, confluent, moist growth. Bact. lipolyticus is greatly favored by the addition of serum to the agar and on such a medium it produces an abundant granular growth. After becoming accustomed to growth on artificial mediums Bact. abortus grows abundantly without the addition of blood serum, and is not especially favored by it.

Since there is such a marked difference in biochemical reactions and cultural characteristics between Bact. lipolyticus and Bact. abortus, it is a fact worthy of attention that Bang's original description of the organism causing contagious abortion agrees with our cultures of Bact. lipolyticus, and does not agree with our cultures of Bact. abortus. Bang noted that the growth of his organism was confined to a zone a few mm. beneath the surface of the agar, that growth in broth culture was in the form of a fine granular sediment, and that growth was greatly favored by the addition of serum to the agar. It would seem by comparing our strains of virulent Bact. abortus with Bang's original description of his organism that there must be more than one variety of bacterium capable of causing abortion in cattle.

In consideration of the probability that typical strains of Bact. abortus are not responsible for every case of abortion among cattle, the bacteriologic findings in the following case are suggestive. Oct. 6, 1917, Cow 27723 of Herd B (Table 2) aborted, and the investigators of the Pathological Division found that her serum reacted negatively to the agglutination test with a suspension of Bact. abortus. They were unable to cultivate Bact. abortus from the pathologic material. Four days after the abortion occurred they submitted to the writer a sample of the uterine exudate, and a sample of milk from this cow. Of the cultures inoculated with the exudate there appeared innumerable colonies on those plates which were incubated in a closed jar with B. subtilis, and on the serum agar plates incubated in ordinary atmosphere. The colonies did not have the characteristic "dew drop" appearance of Bact. abortus, but were the tiny, opaque, compact colonies characteristic of Bact. lipolyticus. Only 2 or 3 of many

colonies transferred to agar slopes grew, and these were cultures which had been incubated in the closed jar with B. subtilis. The growth was scant, in separate colonies, with a granular sediment in the condensation water. In cultural characteristics, therefore, the organism agreed with Bact. lipolyticus. It agreed conspicuously with Bang's description of his organism in its dependence on B. subtilis to render the atmosphere favorable for its growth. It was an exceedingly delicate organism and died 2 or 3 weeks after isolation, before any further study could be made of it.

Table 2 shows that 2 strains of Bact. lipolyticus were obtained from the milk of this cow, and also 3 strains of atypical Bact. abortus. One of them, abd, which was agglutinated in the 1:160 dilution of Bact. abortus antiserum, has already been discussed. Although mindful that the data obtained prove nothing, they are, nevertheless, suggestive in showing that in plate cultures inoculated with the uterine exudate of this cow, which aborted with her serum reacting negatively to the agglutination test with a suspension of Bact. abortus, there appeared innumerable colonies of an organism which differed from our strains of Bact. abortus, but agreed culturally with Bang's original description of the abortion organism; and that the milk of this cow was infected with strains of Bact. lipolyticus which agreed culturally with the strain from the exudate, and also the milk was infected with abortus-like strains, one of which was agglutinated in a 1:160 dilution of Bact. abortus antiserum. Certainly the cow was badly infected with abortion-like organisms, but so far as the investigations of the 2 laboratories could indicate, she was not infected with typical Bact. abortus.

In order to compare our strains of Bact. abortus with the abortion organism recognized in Denmark, a strain was obtained from Professor Bang in Nov., 1916. When received, the agar shake culture had a heavy surface growth and a delicate diaphragm a few mm. beneath the surface. At the time of this writing, 16 months after the culture was received, this strain has not become adapted to growth in ordinary atmosphere, but is dependent on a simultaneous growth of B. subtilis in a closed jar to render the atmosphere favorable for its growth. In this respect it differs from our strains of Bact. abortus. But the growth on agar slope resembles that of our strains, and it also resembles our strains in clouding broth medium. It is agglutinated by the same dilutions of Bact. abortus antiserum as our virulent strains.

It could not be compared with our strains with respect to biochemical reactions, for the ammonia given off by B. subtilis was absorved by the medium, thus masking the changes produced by the Bact. abortus culture. Notwithstanding its continuous dependence on B. subtilis, this strain of Professor Bang resembles our typical strains of Bact. abortus rather than our strains of Bact. lipolyticus.

It is impossible to compare Bact. lipolyticus with Bact. abortus in respect to agglutination reactions, because of the nature of the growth of Bact. lipolyticus. It has been noted that growth in broth cultures appears in the form of a granular sediment. The same granular structure appears also on agar slope cultures. The granules are so compact that vigorous shaking will not break them. On plain agar the growth is too scant to obtain a suspension for the agglutinating test. On serum agar an abundant growth may be obtained, but when passed through filter paper the granules are held back and the filtrate is too clear to serve for the test. It was found that if the suspension was allowed to stand until the largest granules had settled to the bottom, the top could be pipetted off for use in the agglutination test. But such a suspension has so great a tendency to arrange itself in clumps that agglutination in dilutions of the antiserum could never be accurately distinguished.

ATYPICAL STRAINS OF BACT. LIPOLYTICUS

As there are abortus-like bacteria, so there are atypical strains of Bact. lipolyticus. Some of the abortus-like strains vary from the type strain by the possession of properties characteristic of Bact. lipolyticus, and vice versa, some of the atypical strains of bact. lipolyticus vary from the type strain by the possession of properties characteristic of Bact. abortus. The intermediary strains form connecting links between the 2 types, and indicate the relationship between them. Strains xh, xn, xj, and zj (Table 4) are illustrations of abortus-like strains which possess properties characteristic for Bact. lipolyticus. These strains decompose the fat with the formation of acid in the cream layer of litmus whole milk while at the same time they resemble the typical Bact. abortus in rendering alkaline the milk beneath the cream layer. Otherwise these 4 strains could scarcely be distinguished from typical Bact. abortus, except for the fact that they are agglutinated only in the low dilution of 1:40 or 1:80 of the Bact. abortus antiserum.

In the first line of Table 4 under the heading "atypical strains of Bact. lipolyticus" are given the characteristics of a number of strains

which possess the cultural characteristics of the typical Bact. lipolyticus and which vary biochemically from the type strains only in their ability to reduce the hydrogen-ion concentration of broth cultures, a characteristic of Bact. abortus. Also the growth on serum agar was not in such compact granules as is the case with typical Bact. lipolyticus, which made it possible to obtain a suspension suitable for the agglutination test. A suspension of Strain zy, representing this atypical group, was agglutinated in a 1 : 40 dilution of Bact. abortus antiserum.

Altogether there were recognized 5 groups of atypical Bact. lipolyticus, as shown in Table 4. All the groups agreed with the typical strains in cultural characteristics, and varied from the typical strains only slightly in biochemical reactions, the most conspicuous variation being the failure of some of the strains to decompose butter fat.

PREVALENCE OF BACT. LIPOLYTICUS IN COW'S MILK

It has been noted that Bact. lipolyticus was isolated from the milk of 6 of 24, or 25%, of the cows that had not aborted, and from 8 of 12, or 66.6%, of the cows that had aborted, as a result of natural infection, and from 2 of 4, or 50%, of the cows that had aborted after inoculation. (These figures include atypical strains of Bact. lipolyticus.) The numbers per c c were noticeably higher in the milk of cows that had aborted. Judged by these figures, Bact. lipolyticus is apparently inclined to accompany or follow infections of virulent strains of Bact. abortus. It is interesting in this connection to note that the udder of inoculated Cow 50, which is badly infected with Bact. abortus, harbors Bact. lipolyticus in about equal numbers. On the other hand, no Bact. lipolyticus have been found in the material from the udder of Cow 51, which is also badly infected with Bact. abortus.

UDDER FLORA OF A HERD IN WHICH EXISTS AN EPIZOOTIC OF ABORTION

After the data which forms the basis of this report up to this point had been collected, Herd A, which supplied all the samples of milk from cows which had not aborted, became badly infected with Bact. abortus, and an outbreak of abortions occurred, beginning about 3 months after the milk examinations reported in Table 1 were made. Nine of the 12 cows discussed in this paper which had aborted as a result of natural infection (Table 2) belonged also to the same herd, in which there were occasional abortions for several years previous to the outbreak. The milk examinations made in Sept., 1917, as reported in Table 2, were made about 2 months after the outbreak began. During those 2 months, 3 abortions had occurred. Since then several abortions have occurred every month. In the latter part of Jan., 1918, samples of milk for bacteriologic examination were obtained from 12 cows

which had aborted during the preceding 4 months, and at the same time samples of milk were obtained from 9 cows of the same herd which had not aborted. It was thought that the additional data might substantiate the conclusions already drawn. But the results of the examinations revealed so great a change in the udder flora of all the cows whose milk was examined, that it appears to be logical to discuss these results separately instead of including them in Tables 1 and 2.

The results of the bacteriologic examinations are summarized in Table 5. There was no distinction between the bacterial flora of the milk from the cows which had and from those which had not aborted. Therefore all 21 samples will be considered together. There is nothing unusual about the total number of bacteria found in these samples. The percentage of samples in which staphylococci were found agrees essentially with the percentage of a large number of milk samples (192) which were found to contain staphylococci as reported in the earlier paper.[12] The percentage of the 21 samples in which streptococci were found was abnormally high. Eleven of the 21, or 52%, contained streptococci, as compared with an average of 15% of the 192 samples of milk in which streptococci were found. The 192 samples studied in the earlier work were taken from 5 different dairies. The highest percentage of samples containing streptococci from any one dairy was 27. The history of the streptococcic flora of the milk from Herd A is as follows:

In July, 1915, streptococci were found in 5 of 22, or 23% of samples.
In April, 1917, streptococci were found in 7 of 25, or 28% of samples.
In January, 1918, streptococci were found in 11 of 21, or 52% of samples

The figures show that some time during the 9 months preceding the last examination there was a general spread of infection of the udders with streptococci, which resulted in almost doubling the percentage of cows infected, and produced a percentage of more than 3 times the general average as previously determined.

The most conspicuous change in the bacterial flora of the milk from this dairy was the general infection with a streptothrix (Nocardia). Eighteen of the 21, or 85%, of the samples contained this organism. It had spread in the dairy some time during the preceding 4 months, for in the preceding September samples of milk from 6 cows of this dairy had been plated, and no streptothrix was found. The infection of this herd with streptothrix is discussed in an accompanying paper.[12]

The data obtained does not show whether the spread of the streptococcic infection preceded or accompanied the outbreak of abortions. But they show that the streptothrix infection spread after the outbreak had started. It may be that a lowered resistance resulted from the infection with Bact. abortus which prevailed in the herd, and that this lowered resistance permitted the spread of the streptococci and the streptothrix, or the same condition may underlay all 3 abnormal infections.

[12] Jour. Infect. Dis., 1918, 22, p. 373.

TABLE 5

BACTERIAL FLORA OF THE UDDER DURING AN OUTBREAK OF ABORTION. ALL SAMPLES WERE PLATED JAN. 24, 1918

	Cow No.	Date Abortion Occurred	Agglutination Tests		Total No. of Bacteria per C c	No. of Staphylococci per C c	No. of Streptococci per C c	No. of Streptothrix per C c	No. of Bact. Lipolyticus per C c	No. of Abortus-like Bacteria per C c	No. of Acid Producing Rods per C c	No. of Other Bacteria of the Morphology of Bact. Abortus per C c
			Date	Reaction of Blood Serum								
Cows which have aborted during the outbreak	17	10/11/17	11/ 4/16 11/18/17	— +	750	100	0	420	230	0	0	0
	41	12/ 5/17	11/ 4/16 10/18/17	— +	1,100	0	400	200	100	400	0	0
	44	10/13/17	11/ 4/16 10/18/17	— +	2,200	0	600	900	0	0	100	600
	47	1/10/18	11/ 4/16 10/18/17 1/10/18	— — +	2,900	0	500	2,400	0	0	0	0
	49*	1/ 1/18	11/ 4/16 10/18/17 1/ 4/18	— — +	2,500	0	0	1,400	0	0	600	500
	50	11/ 5/17	11/ 4/16 10/18/17	+ +	1,100	200	0	0	0	500	400	0
	53	12/12/17	11/ 4/16 10/18/17 12/13/17	— — +	4,500	0	1,000	3,500	0	0	0	0
	60	1/ 1/18	11/ 4/16 10/18/17	— +	1,500	400	0	900	0	0	200	0
	100†	12/25/17	11/ 4/16 10/18/17	— slight	48,000	0	45,000	3,000	0	0	0	0
	105	11/16/17	11/ 4/16 10/18/17	— —	290	40	0	200	0	0	50	0
	106	1/13/18	10/18/17	+	1,000	500	0	700	0	0	0	0
	110	10/30/17	11/ 4/16 10/18/17	— +	3,150	750	300	1,500	0	0	0	600
Cows which have not aborted during the outbreak	14		11/ 4/16 10/18/17 1/25/18	— — —	1,350	200	150	0	1,000	0	0	0
	54		11/ 4/16 10/18/17 1/25/18	— — slight	1,150	100	200	450	400	0	0	0
	56		11/ 4/16 10/18/17 1/25/18	— — —	1,200	300	0	900	0	0	0	0
	58*		11/ 4/16 10/18/17 1/25/18	— — —	1,060	0	0	280	0	0	500	280
	59		11/ 4/16 10/18/17 1/25/18	— — slight	290	0	0	160	50	0	0	80
	61		11/ 4/16 10/18/17 1/25/18	— — —	1,130	240	90	500	150	150	0	0
	64*		11/ 4/16 1/25/18	— —	3,400	800	0	1,900	0	0	700	0
	201		11/ 4/16 10/18/17 1/25/18	— — slight	36,000	8,000	28,000	0	0	0	0	0
	205		11/ 4/16 10/18/17 1/25/18	— — —	210	0	70	140	0	0	0	0

* The sediment contained red blood corpuscles.
† The sediment was puslike.

It was previously noted that there was no difference between the bacterial flora of the milk of cows which had aborted and those which had not. Bacteria of the morphology of Bact. abortus were isolated from the milk of 66.6% of the cows which had aborted, and from an equal percentage of those who had not aborted. The percentage is lower than in the case of the cows which had aborted as reported in Table 2. But in one case, at least, that of Cow 100, the failure to find organisms related to Bact. abortus can be accounted for by the very large numbers of streptococcus colonies which grew on the plates. Two strains of Bact. lipolyticus were found in the milk of this cow in an examination made 9 months before (Table 1). The same statement applies to Cow 201. For the preceding 4½ years she had been a conspicuous "carrier" of Bact. lipolyticus. It was from her milk that the organism was first isolated, and this was the first examination since then that it failed to grow on the plates. It is most probable that the organism was still eliminated in the milk but that the very great numbers of colonies of streptococci overgrew it. In spite of the failure to isolate abortus-like organisms from Cow 201, bacteria of the morphology of Bact. abortus were isolated from 66.6% of the cows which had not aborted. The percentage is 25% higher than it was for the same herd 9 months previous, indicating that during an outbreak of abortion, the organisms of similar morphology which accompany an infection of the virulent strain spread in the udders of all the cows of the herd.

No typical Bact. abortus could be isolated from any of the 21 samples of milk, although the blood serum of all 12 cows reacted positively to the agglutination test. The failure to isolate them from the milk during an outbreak of abortion strengthens the evidence that the number of virulent Bact. abortus eliminated in cow's milk is not large.

The variation from the typical Bact. abortus was wider in some of the strains with morphology of Bact. abortus which were isolated from these 21 samples of milk than had been found in any of the strains previously studied. Particularly the property of vigorous acid production from the sugars was observed in strains from 7 of the cows. This property had not been observed before in any of the abortus-like organisms which had been studied. In dextrose, maltose, sucrose, and lactose broth cultures with an initial hydrogen-ion concentration of 6.5 P_H, the hydrogen-ion concentration was increased to

about 4.4 P_H. The growth on agar slope resembled Bact. lipolyticus, but in agar shake cultures the colonies were scattered throughout the depth of the agar. On account of these wide variations from the characteristics of Bact. abortus and Bact. lipolyticus the acid-producing rods would not have been considered in connection with those species, except for the facts that 4 of the strains showed the reduction of the hydrogen-ion concentration (about 1.0 P_H) in plain broth culture which is characteristic for Bact. abortus, and the suspensions of 2 of these strains subjected to the agglutination test with Bact. abortus antiserum showed a slight reaction (in the 1:80 or 1:40 dilution). There is no reason to believe they have any pathologic significance, but the fact that they were isolated from 7 of the 21, or 33.3%, of the samples of milk shows that the conditions which permit an infection with Bact. abortus permit also the growth in the udder of a large group of widely varying organisms of similar morphology.

SUMMARY

Bact. lipolyticus and other abortus-like bacteria were isolated from the milk of 10 of 24, or 41.7% of cows which had not aborted. The cows belonged to a herd in which there was an occasional abortion, but no general outbreak.

The same kinds of bacteria were isolated from the milk of 100% of 12 cows which had aborted as a result of natural infection. Bact. lipolyticus was cultivated from the milk of 66.6% of these cows, and other abortus-like bacteria were cultivated from the milk of 50% of them.

Typical virulent strains of Bact. abortus could not be isolated from the milk of either of the groups mentioned above.

Typical Bact. abortus was found to be present in very large numbers in the milk of 2 cows that had been repeatedly inoculated with a mixture of strains of that organism. It was found only once, in rather small numbers (450 per c c) in the milk of a cow which had aborted after being inoculated once with the same mixture of strains of Bact. abortus. It was not found in the milk of another cow which aborted after receiving one inoculation.

The data indicate that virulent strains of Bact. abortus are not eliminated continuously in large numbers in the milk of cows which have aborted, even though the blood serum continues to react positively to the agglutination test.

The characteristics of Bact. lipolyticus and other abortus-like bacteria are described, and their relation to the typical Bact. abortus is discussed. The possibility that some of these strains may cause abortions in those cases in which the blood serum reacts negatively to Bact. abortus antigen is also discussed.

The bacterial flora of the udders of a herd in which there existed an outbreak of abortions was found to be abnormal in the large number of udders which were infected with streptococci, and it was also abnormal in showing a general infection with a streptothrix. Abortus-like bacteria were found in 66.6% of the samples of milk. The abortus-like bacteria included 7 acid-producing strains which had never before been found.